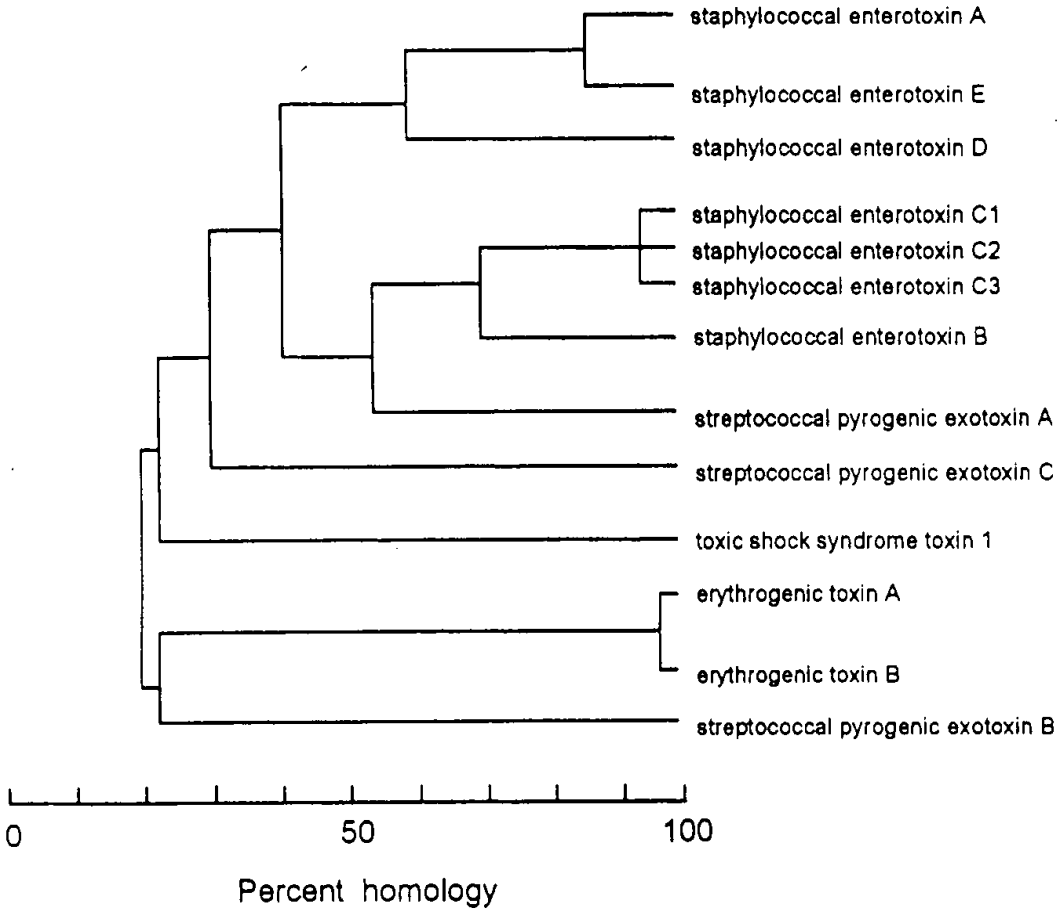
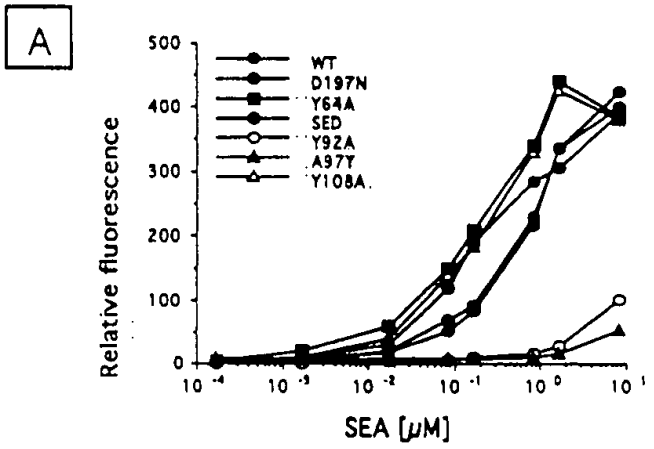
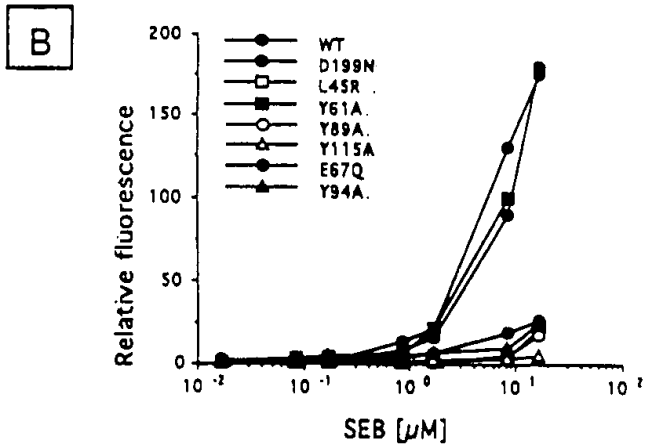

staphylococcal enterotoxin A
staphylococcal enterotoxin E
staphylococcal enterotoxin D
staphylococcal enterotoxin C1
staphylococcal enterotoxin C2
staphylococcal enterotoxin C3
staphylococcal enterotoxin B
streptococcal pyrogenic exotoxin A
streptococcal pyrogenic exotoxin C
toxic shock syndrome toxin 1
erythrogenic toxin A
erythrogenic toxin B
streptococcal pyrogenic exotoxin B
0
50
100
Percent homology

FIGURE 1


A
Relative fluorescence
SEA [μM]
WT
D197N
Y64A
SED
Y92A
A97T
Y108A.


B
Relative fluorescence
SEB [μM]
WT
D199N
L45R
Y61A.
Y89A.
Y115A
E67Q
Y94A.

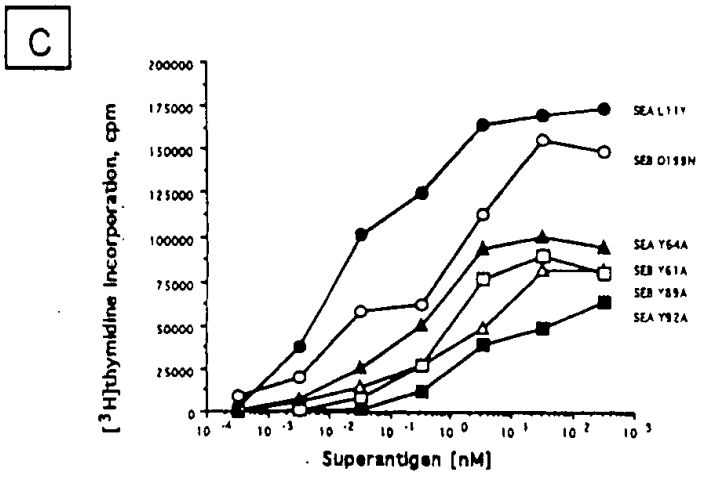

C
[3H]thymidine incorporation, cpm
Superantigen [nM]
SEA Y64A
SEB Y61A
SEB Y89A
SEA Y92A

Fig. 2

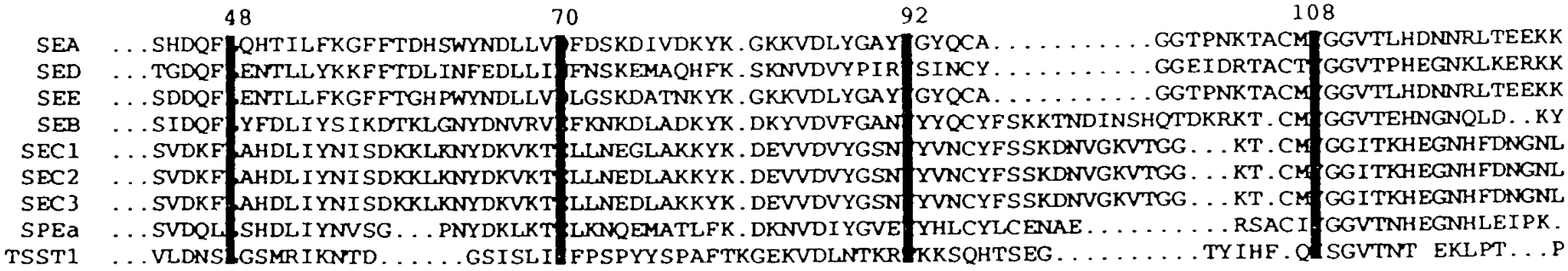

48
70
92
108
SEA ...SHDQFLQHTILFKGFFTDHSWYNDLLVFDSKDIVDKYK.GKKVDLYGAYIGYQCA..........GGTPNKTACMIGGVTLHDNNRLTEEKK
SED ...TGDQFLENTLLYKKFFTDLINFEDLLIFNSKEMAQHFK.SKNVDVYPIRISINCY..........GGEIDRTACTIGGVTPHEGNKLKERKK
SEE ...SDDQFLENTLLFKGFFTGHPWYNDLLVLGSKDATNKYK.GKKVDLYGAYIGYQCA..........GGTPNKTACMIGGVTLHDNNRLTEEKK
SEB ...SIDQFLYFDLIYSIKDTKLGNYDNVRVEFKNKDLADKYK.DKYVDVFGANYYQCYFSKKTNDINSHQTDKRKT.CMIGGVTEHNGNQLD..KY
SEC1 ...SVDKFLAHDLIYNISDKKLKNYDKVKTELLNEGLAKKYK.DEVVDVYGSNYYVNCYFSSKDNVGKVTGG...KT.CMIGGITKHEGNHFDNGNL
SEC2 ...SVDKFLAHDLIYNISDKKLKNYDKVKTELLNEDLAKKYK.DEVVDVYGSNYYVNCYFSSKDNVGKVTGG...KT.CMIGGITKHEGNHFDNGNL
SEC3 ...SVDKFLAHDLIYNISDKKLKNYDKVKTELLNEDLAKKYK.DEVVDVYGSNYYVNCYFSSKDNVGKVTGG...KT.CMIGGITKHEGNHFDNGNL
SPEa ...SVDQLLSHDLIYNVSG...PNYDKLKTELKNQEMATLFK.DKNVDIYGVEYYHLCYLCENAE..........RSACIIGGVTNHEGNHLEIPK.
TSST1 ...VLDNSLGSMRIKNTD......GSISLIIFPSPYYSPAFTKGEKVDLNTKRYKKSQHTSEG..........TYIHF.QISGVTNT EKLPT...P

Fig. 3

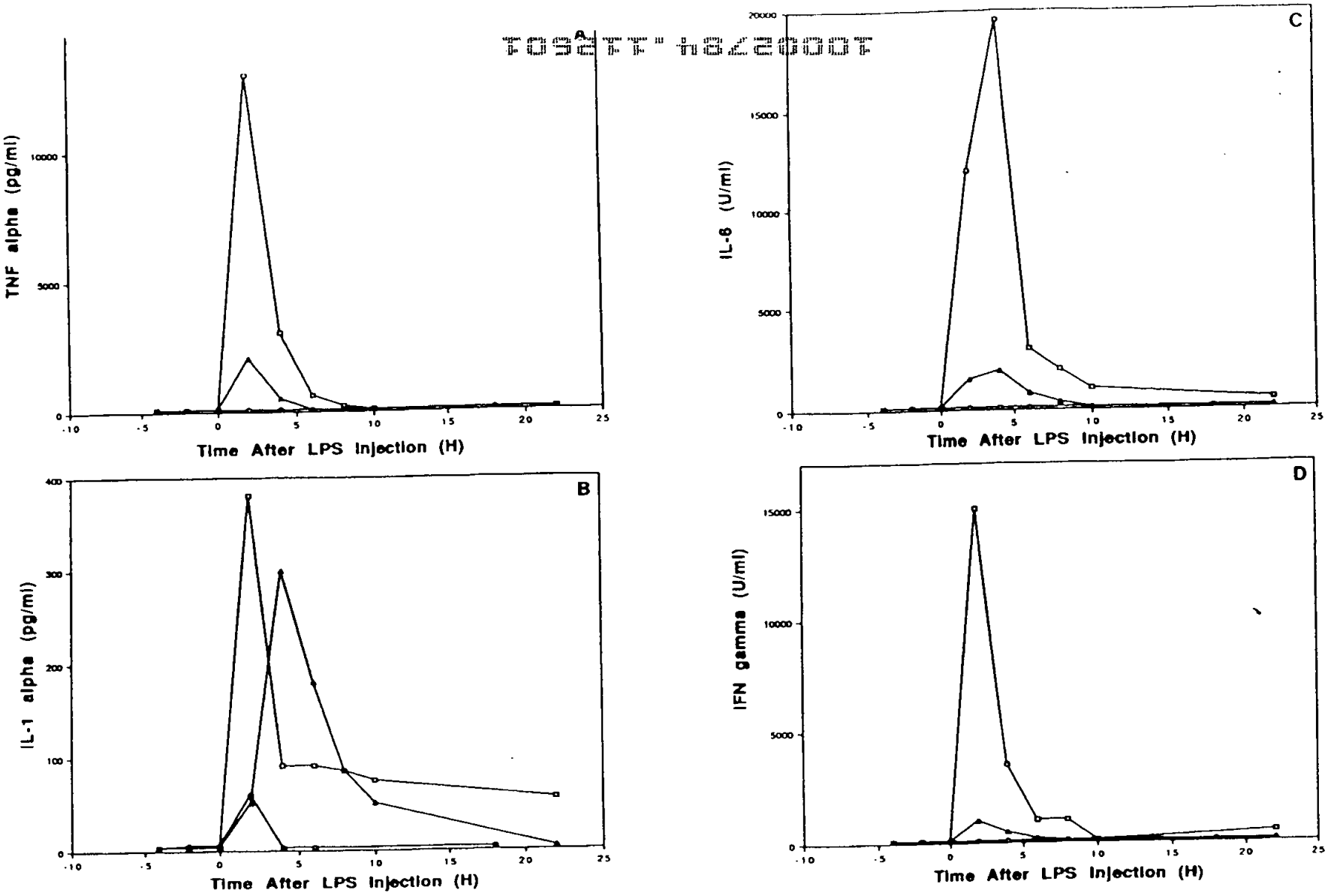

A
TNF alpha (pg/ml)
Time After LPS Injection (H)
B
IL-1 alpha (pg/ml)
Time After LPS Injection (H)
C
IL-6 (U/ml)
Time After LPS Injection (H)
D
IFN gamma (U/ml)
Time After LPS Injection (H)

Fig 4

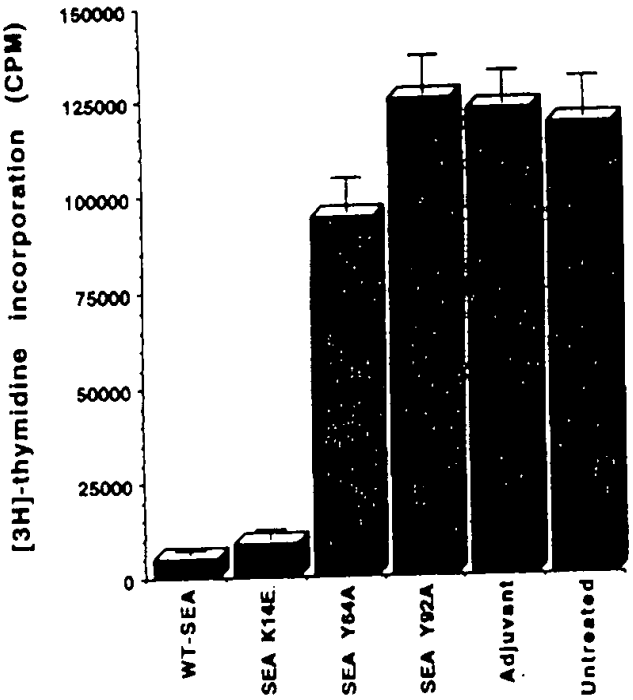
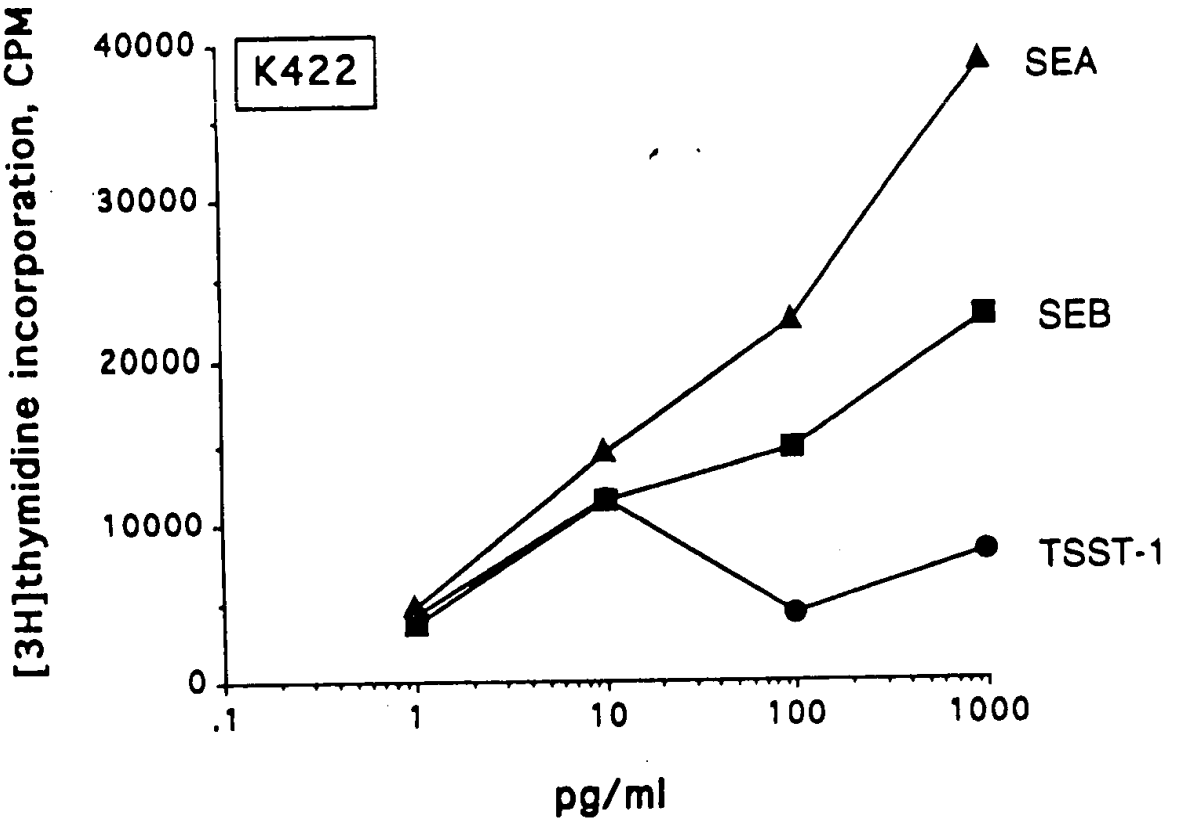

[3H]-thymidine incorporation (CPM)
150000
125000
100000
75000
50000
25000
0
WT-SEA
SEA K14E
SEA Y64A
SEA Y92A
Adjuvant
Untreated

Fig. 5


K422
[3H]thymidine incorporation, CPM
40000
30000
20000
10000
0
.1
1
10
100
1000
pg/ml
SEA
SEB
TSST-1

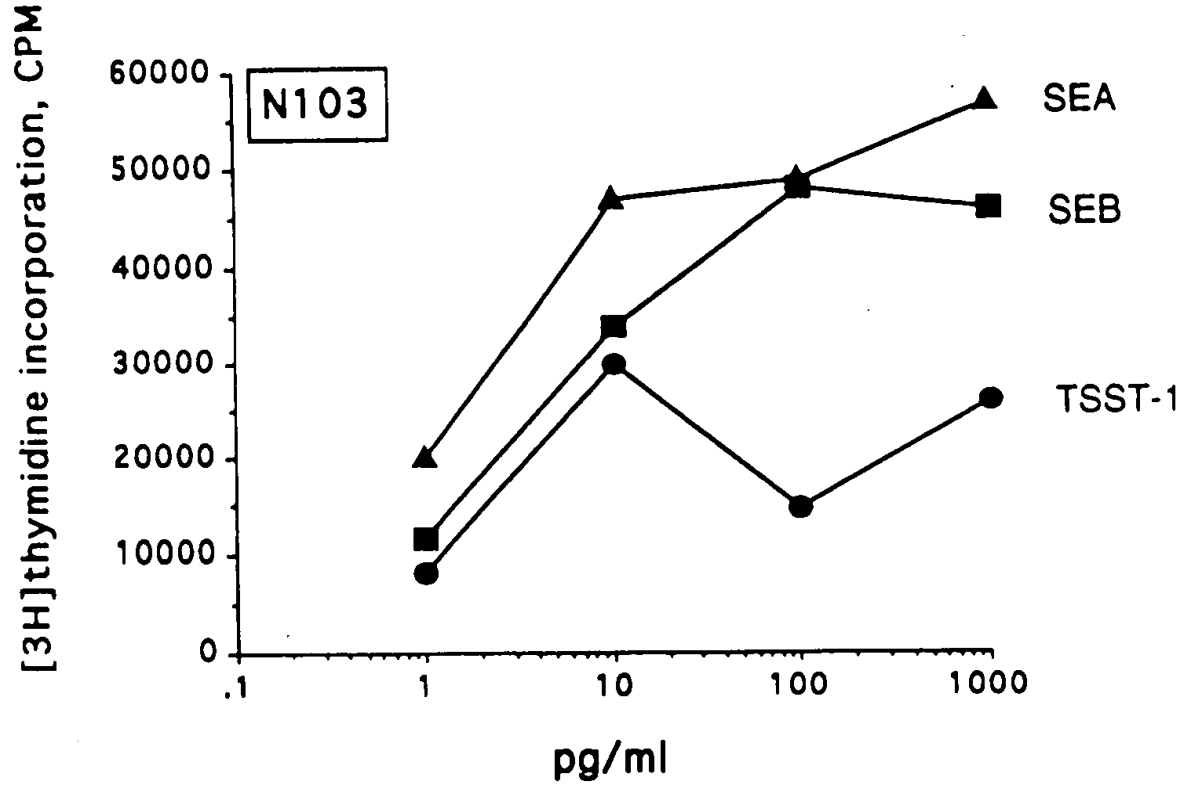
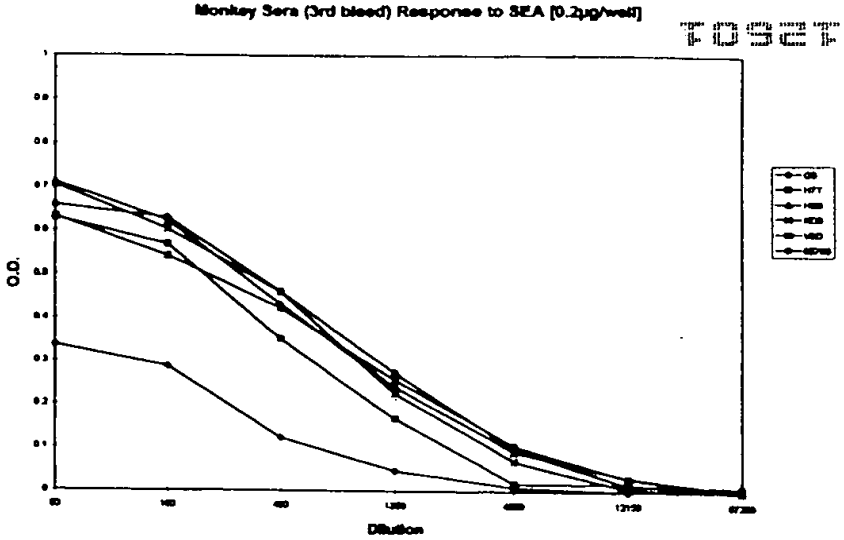

N103
[3H]thymidine incorporation, CPM
60000
50000
40000
30000
20000
10000
0
.1
1
10
100
1000
pg/ml
SEA
SEB
TSST-1

Fig. 6


Monkey Sera (3rd bleed) Response to SEA [0.2µg/well]
O.D.
Dilution

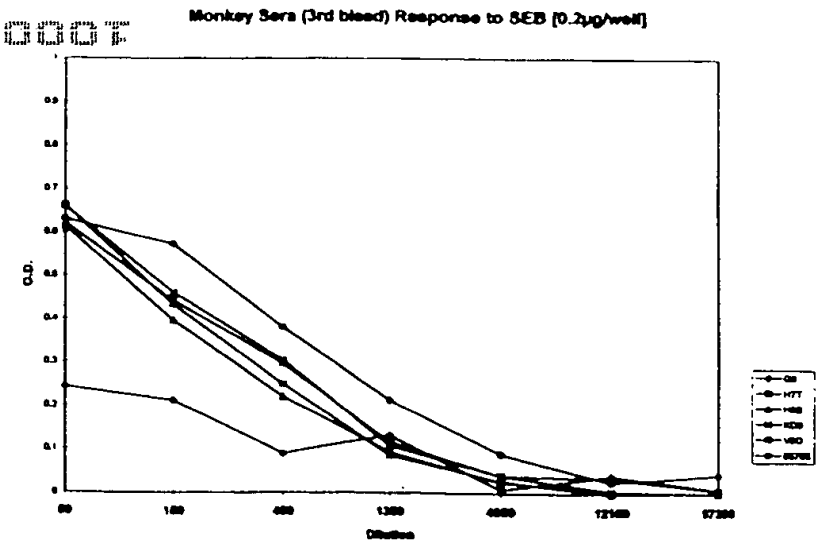
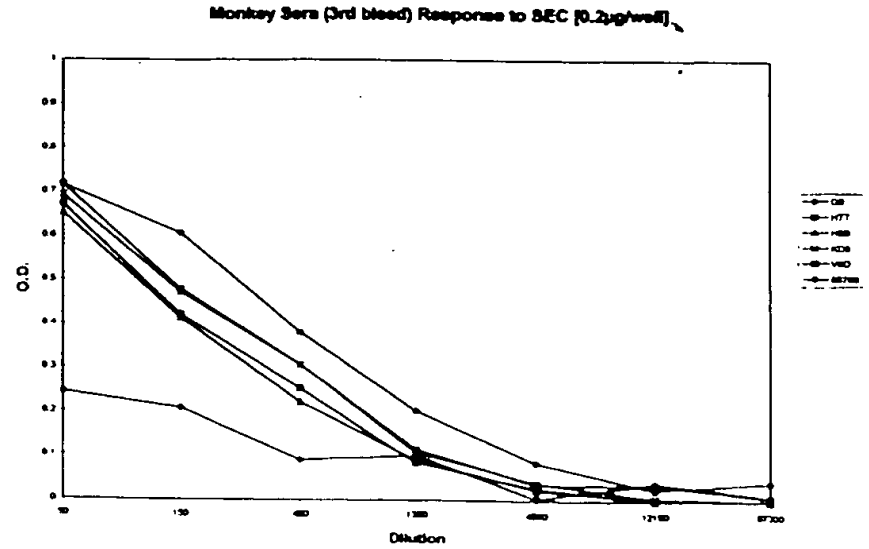
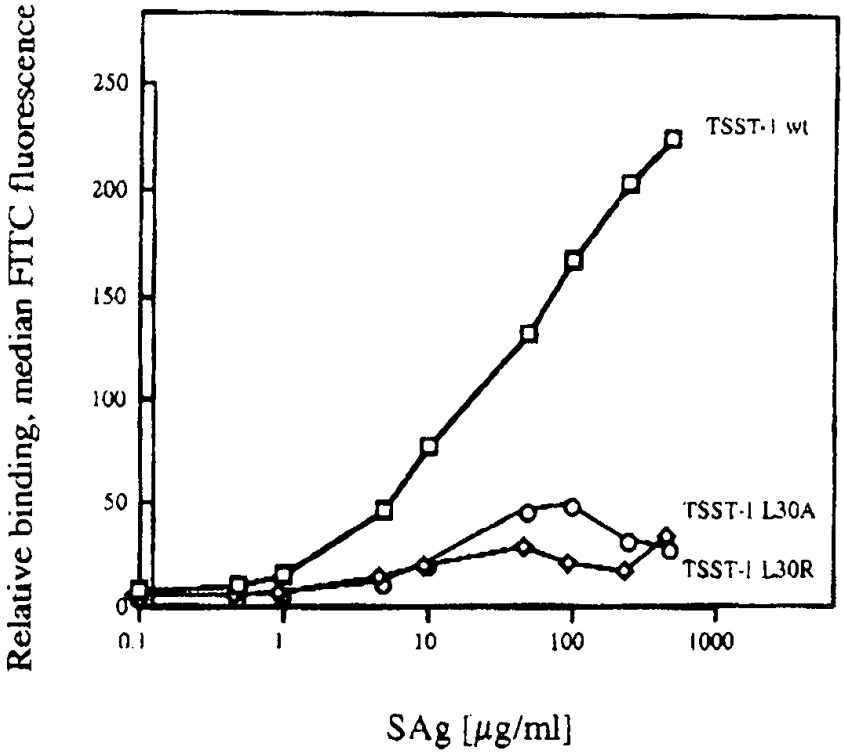

Monkey Sera (3rd bleed) Response to SEB [0.2µg/well]
O.D.
Dilution

Fig. 7


Monkey Sera (3rd bleed) Response to SEC [0.2µg/well]
O.D.
Dilution

A.


Relative binding, median FITC fluorescence
250
200
150
100
50
0
0.1
1
10
100
1000
TSST-1 wt
TSST-1 L30A
TSST-1 L30R
SAg [μg/ml]

B.

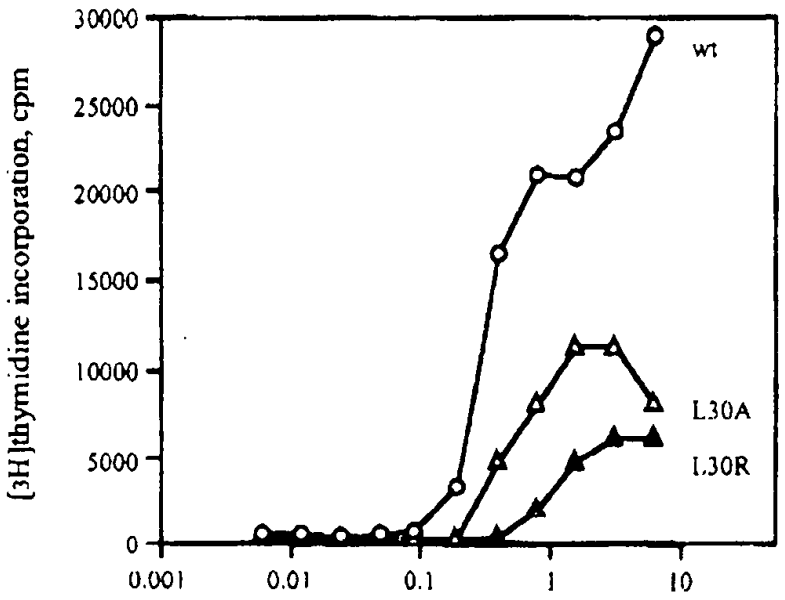
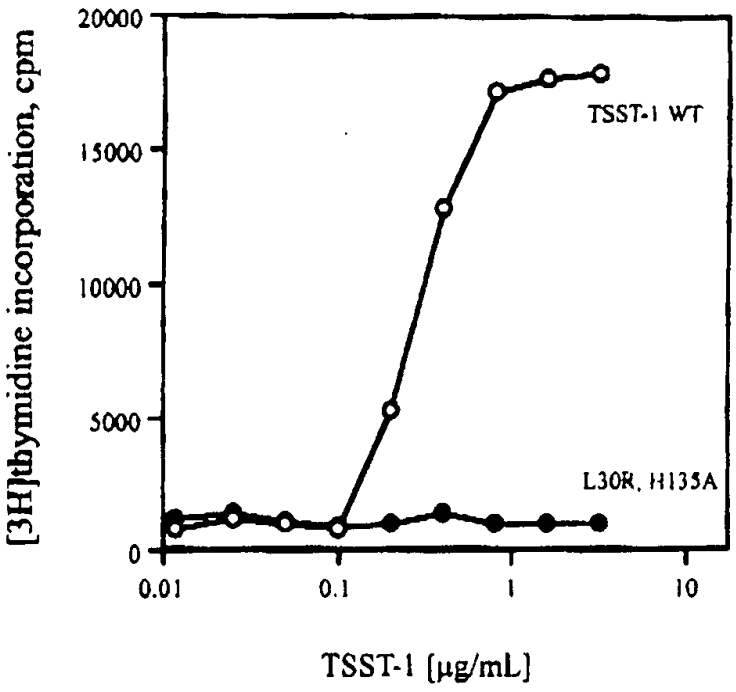

[3H]thymidine incorporation, cpm
30000
25000
20000
15000
10000
5000
0
0.001
0.01
0.1
1
10
wt
L30A
L30R
TSST-1 [μg/mL]

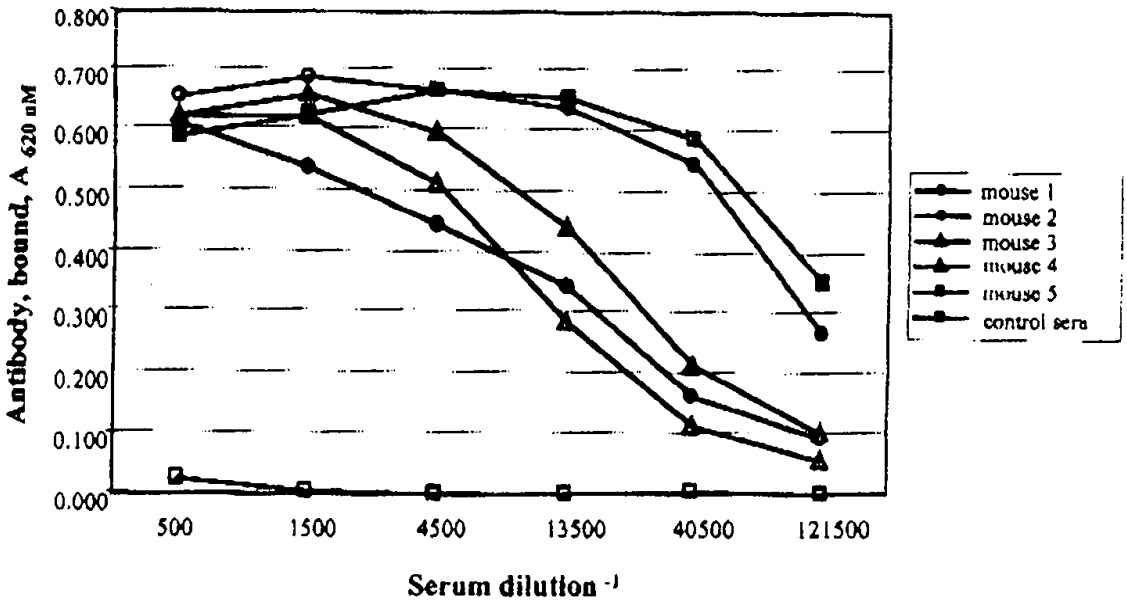
FIGURE 8

C.



Biological activities of TSST-1 mutants. a, Mutations of TSST-1 at amino acid position 30 (L30R, L30A) results in greatly diminished interactions with cell surface HLA-DR, measured by laser fluorescence-activated flow cytometry and FITC-labeled rabbit anti-TSST-1 antibody (affinity purified). b, Mutations of TSST-1 at amino acid position 30 (L30R, L30A) results in greatly diminished activation of human lymphocytes; c, Introduction of an additional mutation, H135A to the TSST-1 mutant L30R results in the maximum reduction in T-cell stimulation. Human T-cell proliferation, was assessed by [3H]thymidine incorporation, using a 12 h pulse with label and harvesting cells after 60 h of culture. Each data point represents the mean of triplicate determinations; SEM <5%.

FIGURE 8



Antibody response to TSST-1 mutant L30R. Mice received a total of three injections of vaccine (20 μg/mouse) in Alhydrogel, two weeks between injections. Sera were sampled two weeks after last vaccination and anti-TSST-1 specific antibody was measured by ELISA, using plates coated with wild-type TSST-1. Pooled non-immune mouse sera were used as negative control.

FIGURE 9

A.

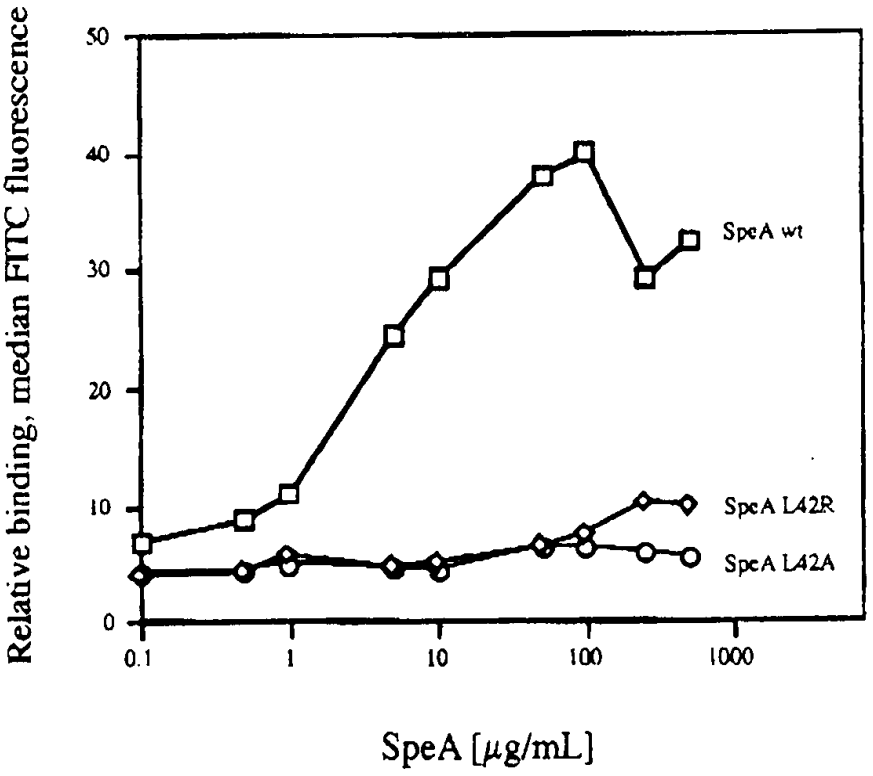
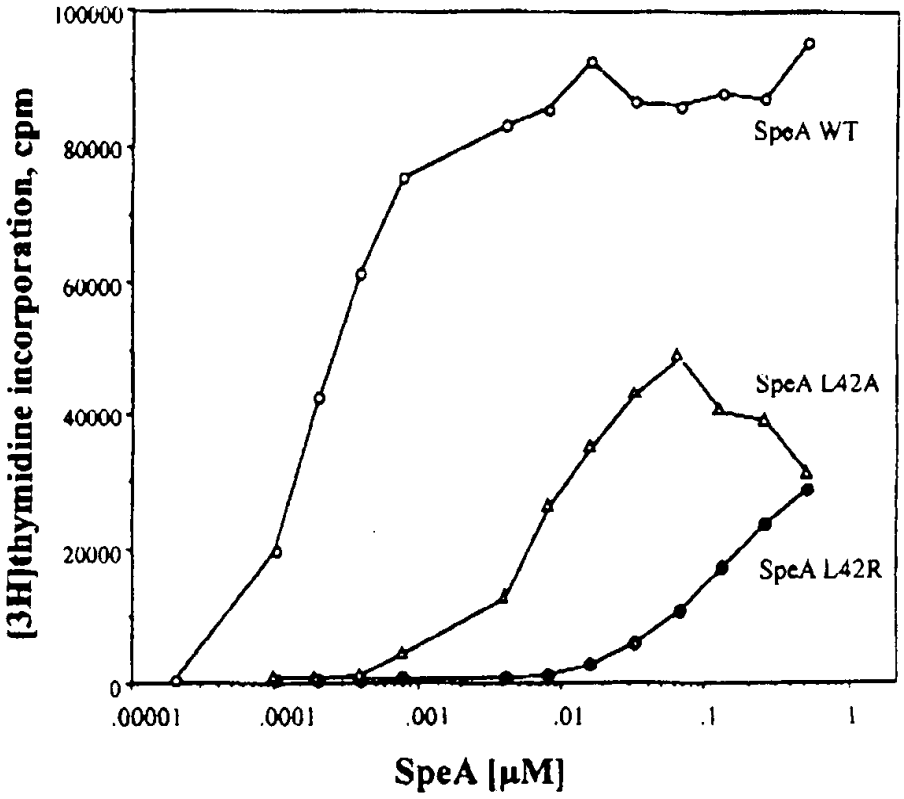

Relative binding, median FITC fluorescence
50
40
30
20
10
0
0.1
1
10
100
1000
SpeA wt
SpeA L42R
SpeA L42A
SpeA [μg/mL]

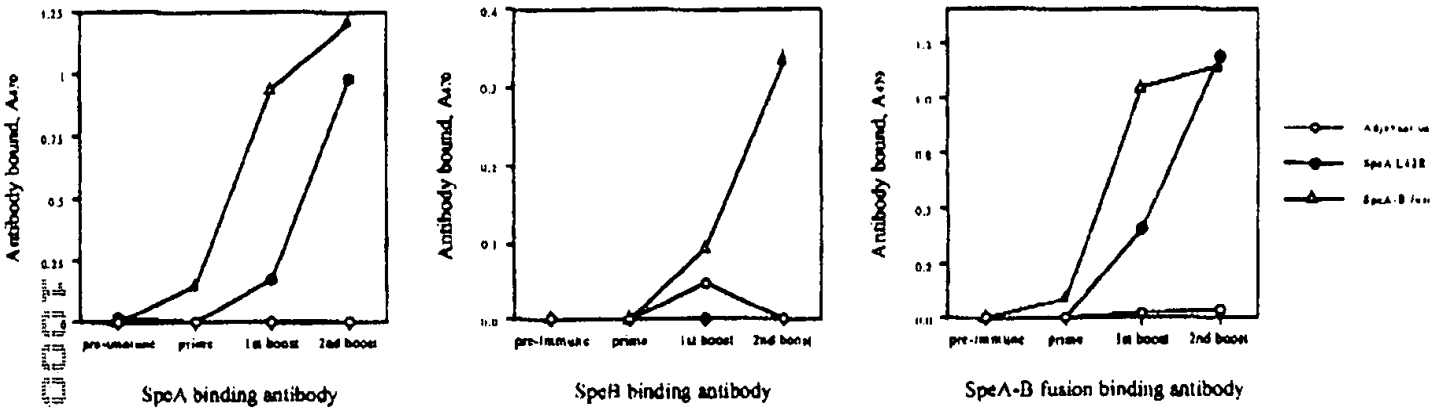
FIGURE 10

B.



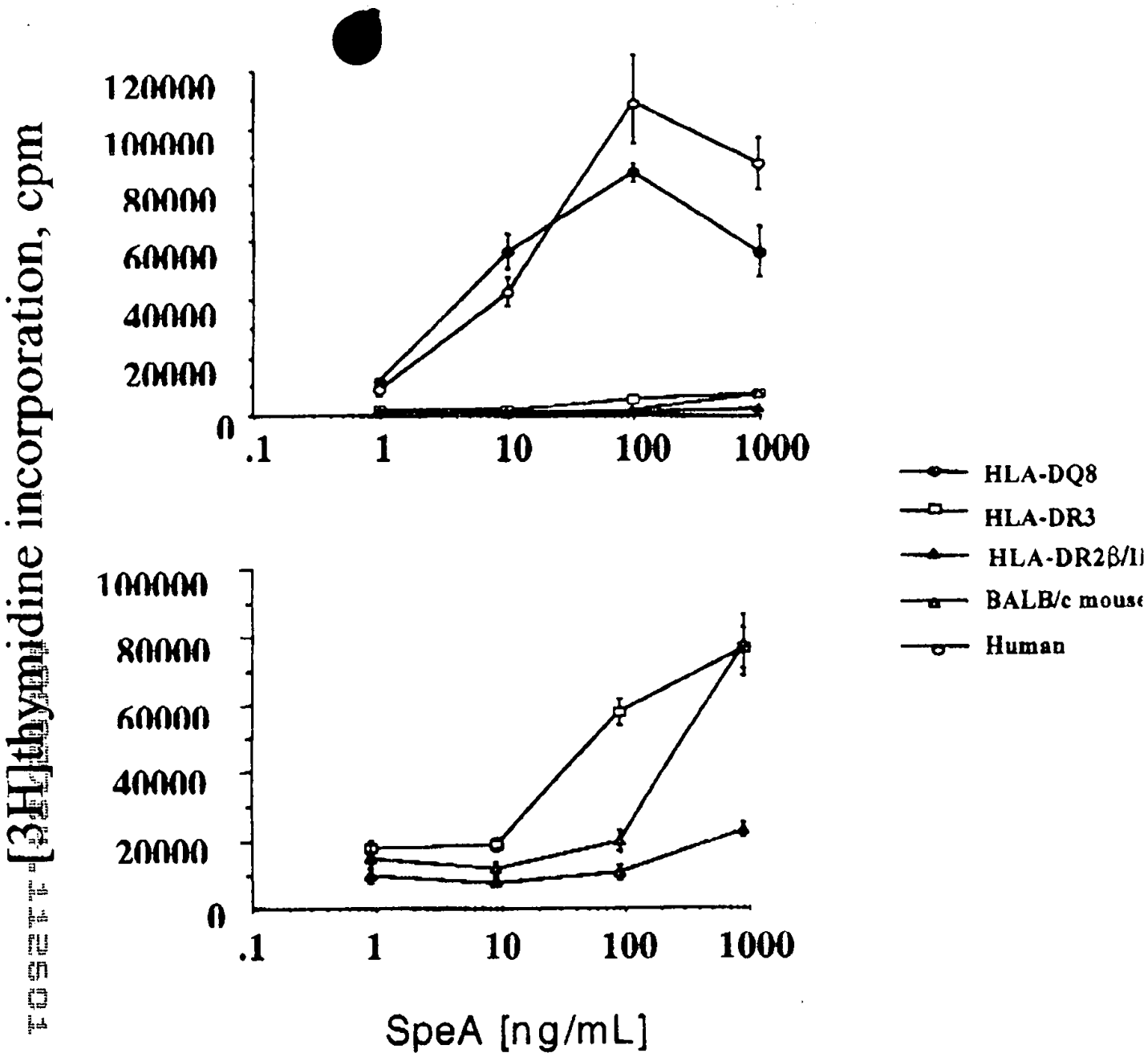
Biological activities of SpeA mutants. a, Mutations of SpeA at amino acid position 42 (L42R) results in greatly diminished interactions with cell surface HLA-DR, measured by laser fluorescence-activated flow cytometry and FITC-labeled rabbit anti-SpeA antibody (affinity purified). b Mutations of SpeA at amino acid position 42 (L42R or L42A) results in greatly diminished activation of human lymphocytes. Human T-cell proliferation, was assessed by [3H]thymidine incorporation, using a 12 h pulse with label and harvesting cells after 60 h of culture. Each data point represents the mean of triplicate determinations; SEM <5%.

FIGURE 10



Mouse antibody response to SpeA L42R and SpeA-B fusion constructs. BALB/c mice were vaccinated three times with 10 µg plus adjuvant (MPL™ + TDM+ CWS Emulsion, RIBI ImmunoCHem Research, Inc., Hamilton, MT) of each construct, allowing two weeks between injections. Sera from each experimental group (n=5) were pooled for measurement of specific antibodies. Data shown are antigen-specific antibodies (ELISA units) present in a 1:100,000 dilution of pooled sera from mice vaccinated with SpeA L42R, SpeA-B fusion or adjuvant only.

FIGURE 11



T-cell response *in vitro* of mononuclear cells from transgenic mice expressing HLA-DQ8αβ and human CD4 closely approximate the physiological response of humans. Mononuclear cells were isolated from spleens of transgenic mice expressing HLA-DR3, HLA-DQ8 or HLA-DR2β/IEα, or non-transgenic BALB/c mice and human peripheral blood (4 x 105/well). Following 60 h culture with SpeA, cells were pulse-labeled (12 h) with 1 μCi of [3H]thymidine. DNA from cells was harvested onto fiberglass filters and incorporated radioactivity measured by liquid scintillation.

FIGURE 12